Doctoral thesis/dissertation Digest Form

Thesis/dissertation Title: *In vivo* photo-crosslinking combined with genetic analysis to elucidate the substrate recognition mechanism of TOR complex 2

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Target of Rapamycin (TOR) protein kinase forms two distinct multi-protein complexes, termed TOR Complex 1 (TORC1) and 2 (TORC2). These complexes respond to different environmental stimuli transmitted by specific activator proteins and interact different substrates to regulate them through phosphorylation. Thus, protein-protein interactions play crucial roles in the signal-mediated regulation of the TOR complexes as well as in their execution of the specific cellular functions.

One of the techniques to capture dynamic *in vivo* protein-protein interactions is to introduce photo-reactive, unnatural amino acids into a specific position of a protein of interest for photo crosslinking analysis. I had demonstrated that site-directed *in vivo* photo crosslinking technique can be applied to the fission yeast *Schizosaccharomyces pombe*, a popular model organism for the study of cellular mechanisms that are conserved among eukaryotes.

Subsequently, the technique has been utilized to characterize the interaction between the TORC2 subunit Sin1 and the TORC2 substrate Gad8. Although the Sin1 CRIM domain was previously shown to interact with Gad8, the molecular interface between CRIM and Gad8 was not known. My analysis using the *in vivo* photo-crosslinking technique has suggested that the protruding acidic loop of the CRIM domain and subdomain I of the Gad8 kinase are required for the Sin1 CRIM-Gad8 interaction.

The genetic and biochemical approaches to analyze subdomain I of the Gad8 kinase revealed the critical residues required for the interaction with Sin1 CRIM. I propose that the hydrophobic interaction between the conserved hydrophobic residues in the acidic loop of Sin1 CRIM and the identified hydrophobic residues in subdomain I of the Gad8 kinase enables Sin1 CRIM to recruit Gad8 toward TORC2. This result offers a new insight into the mechanism by which the Sin1 subunit of TORC2 specifically recognizes the AGC-family protein kinase Gad8. Because both Sin1 CRIM and Gad8 signaling are highly

conserved among eukaryotes, this regulatory system possibly exists in other organisms including human.

In parallel to the above project, I have attempted to apply the *in vivo* photo-crosslinking technique to determine how the Rhb1 GTPase interacts with TORC1 in fission yeast. The Rheb GTPase is an established activator of mTORC1, but the direct interaction of Rheb with mTORC1 was not demonstrated when this study was initiated. By expressing Rhb1, a fission yeast Rheb homolog, with pBpA incorporated, proteins that interact with Rhb1 have been sought by the *in vivo* photo-crosslinking technique. Although the identity of the proteins crosslinked to Rhb1 could not be determined, my experiments have identified the suitable conditions for the *in vivo* incorporation of pBpA into the Rhb1 protein and the feasibility of this technique in capturing novel *in vivo* protein-protein interactions in fission yeast.